

HAEMOLYMPH COMPOSITION IN NAMIB DESERT TENEBRIONID BEETLES

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Abstract—1. Haemolymph composition was investigated in eight species of Namib Desert tenebrionid beetles.

2. In general, haemolymph volume correlated positively with total body water. No consistent interspecies differences were observed.

3. The effects of mild dehydration on haemolymph composition were investigated in four species. The results indicate haemolymph dilution as a possible initial osmoregulatory response.

4. Haemolymph composition from *L. discoidalis* specimens collecting water during a fog was not significantly different from that of specimens foraging prior to a fog. This indicates that osmotic stress may not be the only reason for collecting fluid under these conditions.

INTRODUCTION

The Namib Desert has an abundant arthropod fauna including the long-lived tenebrionid beetles. A vital source of liquid water in the west is irregularly occurring fog and certain of these beetles have developed a variety of mechanisms to utilize this. Fog condensate is taken up from wet sand, from vegetation and by fog basking (Seely, 1979). For other beetles, no specific types of water uptake behaviour have been described, although they probably utilize one of the above or take up water directly from the vapour phase (Cooper, 1982). Water uptake behaviour is to some extent correlated with habitat (Seely, 1979; Seely *et al.*, 1983).

Very little is known concerning haemolymph composition in the Namib Desert tenebrionids, although information is available relating to species found in other deserts (Broza *et al.*, 1976; Coutchie and Crowe, 1979; Riddle *et al.*, 1976). Of particular interest is whether haemolymph composition may be correlated with fog-basking (*Onymacris unguicularis*) or trench-digging (*Lepidochora discoidalis*) behaviour (Seely, 1979). The only studies which have been done on haemolymph composition are by Nicolson (1980) and Cooper (1982) on aspects of the water relations of *O. plana* and *O. unguicularis*; the former investigating the effects of dehydration and subsequent hydration on laboratory-acclimated animals, and the latter reporting on water-balance including osmolality values (from haemolymph obtained from freshly caught field animals). In view of this paucity of information, a comparative study, using beetles from different habitats, was undertaken in order to establish whether haemolymph composition in tenebrionid beetles was species and/or habitat dependent. In addition, the effects of mild dehydration on certain species was investigated with a view to relating differences in response, if any, to specific water-uptake behaviour. In this regard it is known (at least for some beetles) that a 10–12-day dehydration period will result in haemolymph concentration, and that the

beetles will then drink water in the laboratory (from droplets) (Seely and Hamilton, 1976; Nicolson, 1980). However, a lesser dehydration stress, as envisaged in this study, could yield more information about the mechanisms involved. Finally, haemolymph composition from *L. discoidalis* specimens, caught "trench digging" during a fog, was compared to that from specimens obtained from the same area prior to the fog.

MATERIALS AND METHODS

Adult beetles were collected in the sand dunes, interdune valleys or dry river beds at or near Gobabeb, South West Africa. The species used, habitat and reported method of water uptake are indicated in Table 1. They were either used immediately or dehydrated for two days in a desiccator over silica gel (10–15% RH) at the laboratory in Gobabeb (23°C). All beetles were weighed to the nearest 0.1 mg before experimentation. Haemolymph samples were collected from the coxa or directly from the dorsal vessel (after careful removal of the elytra) into capillary tubes. Haemolymph volume was determined according to the gravimetric method of Richardson *et al.* (1931). Total body water was determined by subtracting dry weight (after 48 hr at 70°C) from the wet weight. Unless stated otherwise, individual samples were analysed for osmolality (Wescor 5120B vapour pressure osmometer), chloride, sodium and potassium concentrations (Radiometer CMT 10 chloride titrator for chloride and FLM 3 flame photometer for sodium and potassium), colloid osmotic pressure (electronic osmometer after Prather *et al.*, 1968), and for total protein (according to the method of Lowry *et al.*, 1951) using BSA as standard. The first two analyses were done (immediately) at Gobabeb whilst the others were done on samples (from the same beetle) flown to Johannesburg in sealed (Cristaseal) capillary tubes. Results were compared statistically using Student's *t*-test and non-parametric analyses (ANOVA).

RESULTS

Haemolymph composition in normal field caught beetles

The results are shown in Table 2. It was possible to distinguish between males and females on the basis

Table 1. Species used, habitat and reported mechanism of water uptake during or after a fog (Seely, 1979)

Species	Habitat	Water obtained from
<i>Onymacris unguicularis</i>	Vegetationless dunes	Fog basking
<i>Lepidochora discoidalis</i>	Dune slipfaces	Trench digging (sand)
<i>Onymacris plana</i>	Dune base and interdune valley (vegetation present)	Vegetation (detritus)
<i>Stenocara gracilipes</i>	Sand of dry river bed on edge of dune field	Sand?
<i>Onymacris rugatipennis</i>	Sand of dry river bed on edge of dune field	Vegetation?
<i>Physodesmia globosa</i>	Sand of dry river bed on edge of dune field	Vegetation?
<i>Physosterna cribripes</i>	Dry river bed in gravel plain	?
<i>Stips stali</i>	Plinth area of dune	?

of morphological features except in the cases of *L. discoidalis* and *S. stali*. For some analyses, insufficient volume was available and the remaining samples were either pooled or no distinction was drawn between the sexes.

The results shown in Table 2 indicate that no significant differences were present in haemolymph composition of males and females (except chloride concentration and osmolality in *O. plana*). Females tended to show higher mean osmolalities than males and in all species in general, whenever eggs were present higher values for this variable were recorded (again not statistically significantly different). Also, females had higher body masses, haemolymph volumes and total body water contents. In both males and females, for all species except *O. unguicularis* and *S. gracilipes*, haemolymph volume correlated significantly ($\alpha = 0.05$) with total body water (*O. plana*, $r = 0.802$ (m) and $r = 0.943$ (f); *O. rugatipennis*, $r = 0.830$ (f); *P. globosa*, $r = 0.977$ (m) and $r = 0.924$ (f); *P. cribripes*, $r = 0.997$ (m) and $r = 0.918$ (f); *S. stali*, $r = 0.774$; and *L. discoidalis*, $r = 0.773$). Total protein correlated with C.O.P. at $r = 0.71$ for *O. unguicularis* ($\alpha = 0.05$), $r = 0.67$ for *O. rugatipennis* and $r = 0.92$ for *P. cribripes* ($\alpha = 0.01$).

Interspecies differences were few. Females of *O. plana* and *S. gracilipes* showed significantly greater osmolalities than those of the other species ($P < 0.05$). Chloride concentrations in *O. plana* females were significantly greater than those of females from the other species, and total protein concentration in *O. unguicularis* significantly lower when compared to the other values ($P < 0.05$).

Effects of mild dehydration

Haemolymph composition in four species of desert tenebrionids is shown in Table 3. When compared to the results shown in Table 2, it is clear that few statistically significant differences were present except for sodium concentration which decreased in most cases ($P < 0.05$). The general trend, in the cases of osmolality and potassium concentration, also seemed to be decreased values. Haemolymph volume, expressed as a percentage of initial body mass, decreased, remained the same or increased. Interspecies differences were again few. Female *P. cribripes* sodium concentrations were significantly higher ($P < 0.05$) when compared to the other species (except for *O. unguicularis*), and *O. unguicularis* potassium concentrations were less than that found in females from *P. cribripes* ($P < 0.05$).

Effects of hydration

L. discoidalis beetles were collected from a dune

during a fog. The beetles were all in the process of collecting water by means of "trench digging". Table 4 shows the results obtained.

Osmolality correlated negatively with sodium concentration ($r = -0.728$; $\alpha = 0.01$). It is evident that haemolymph dilution occurred because haemolymph volume increased and all other variables measured decreased compared to those from beetles caught before the fog. None of the differences recorded, however, were statistically significant. Most animals were lethargic after taking up water but recovered within an hour or two.

DISCUSSION

A comparison with the results of Nicolson (1980) for *O. plana* and of Cooper (1982) for *O. unguicularis* indicates that different mean values were found for most variables here. A seasonal variation is possible, as was found by Riddle *et al.* (1976) for a tenebrionid larva from a different desert. Another possibility is that although environmental conditions may have been similar, food availability and micro-climate may not have been the same. The variability reported by Nicolson (1980) for *O. plana* in a laboratory study is less than we found. If the beetles are sensitive to such factors as food availability and micro-climate, this may be reflected in (large) variations in haemolymph composition which could be tolerated. In this regard we have found haemolymph composition in beetles transported to the laboratory in Johannesburg (and acclimated for 3–4 weeks on a diet of oatmeal and lettuce) different to that reported here (unpublished). A third possibility is that the presence or absence of eggs (and sperm?) may have influenced the results. It was mentioned before that in general higher osmolalities were recorded when eggs were present, although no definite relationship with regard to the stage of egg development could be established. It is possible that the production of eggs (and sperm?) is experienced as a dehydration stress by the beetles because a fair amount of fluid is required (eggs are claimed to be 90% water by mass; Cooper, 1982).

The values reported here for colloid osmotic pressure require special mention. Positive correlations were found with protein concentration, and dilution experiments have indicated a similar curvilinear relationship to that which exists between, for instance, albumin and C.O.P. (Prather *et al.*, 1968). However, in the case of mammalian fluid, the measured C.O.P. remains constant once the plateau value is reached. With the haemolymph used here, the C.O.P. decreased after an initial spike value, and reached a plateau a few minutes later. Bearing in mind that the

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Table 2. Haemolymph composition in normal field-caught Namib Desert tenebrionid beetles

Variable		<i>O. unguicularis</i>		<i>O. rugatipennis</i>		<i>P. globosa</i>		<i>P. cribripes</i>		<i>O. plana</i>		<i>L. discoidalis</i>	<i>S. stali</i>	<i>S. gracilipes</i>	
		M	F	M	F	M	F	M	F	M	F			M	F
Osmolality	\bar{x}	363.6	366.0	393.8	409.2	389.0	416.8	409.0	393.0	400.0	463.3*	387.8	402.3	393.7	432.0
(mosm/kg)	SD	15.7	29.0	19.3	29.5	19.1	44.1	35.8	26.8	18.7	40.4	13.7	37.0	28.7	26.0
Cl	\bar{x}	115.0	119.8	120.1	117.4	110.6	116.4	120.0	110.8	110.5	134.3*	121.7	111.2	103.4	105.0
(mmol/l)	SD	4.2	7.9	7.4	14.7	5.7	6.9	7.8	10.5	13.2	11.7	10.2	6.2	20.4	23.0
Na	\bar{x}	146.7	134.3	160.3	141.4	135.8	143.2	137.3	144.6	141.5	150.9	138.0	—	128.8	
(mmol/l)	SD	15.4	20.6	12.8	10.5	16.7	7.7	14.9	9.2	29.9	23.3	10.5	—	17.3	
K	\bar{x}	18.3	18.9	21.1	18.0	24.0	22.8	18.3	16.2	18.4	25.9	21.3	—	21.8	
(mmol/l)	SD	3.0	4.9	5.2	2.9	6.4	5.3	2.2	4.1	3.2	6.1	4.8	—	3.9	
Total protein	\bar{x}		12.8		38.8		36.9		27.5		33.9	40.5	—	—	
(g/l)	SD		5.9		2.4		6.2		6.1		11.7	(pooled)	—		
C.O.P.	\bar{x}	5.3	5.6		7.1		10.1		8.4		11.0	7.8	—	9.9	
(mmHg)	SD	1.1	2.4		2.4		2.2		0.8		2.1	(pooled)		(pooled)	
Body mass	\bar{x}	521	597	459	870*	512	1036*	791	1428*	797	853	82	118	216	40*
(mg)	SD	47	49	78	229	130	116	134	118	112	182	20	14	31	1
Total body	\bar{x}	365	418*	282	570*	299	653*	482	964*	467	536	50	62	108	24*
water (mg)	SD	32	6	39	159	84	107	87	119	121	171	16	11	31	1
Haemolymph	\bar{x}	120	135	63	141*	70	214*	127	366*	125	222	30	29	42	7*
volume (μ l)	SD	17	19	17	55	32	107	20	114	85	175	11	8	17	1
Haemolymph		23	23	14	16	14	21	16	26	16	26	37	25	19	1
volume (% BM)															
Total body		70	70	62	66	59	63	61	68	59	63	62	53	50	6
water (% BM)															
N		5	5	7	9	5	5	5	5	9	7	15	10	6	

*Indicates a significant difference between males and females ($P < 0.05$).

Table 3. Haemolymph composition in four species of desert tenebrionids dehydrated for two days at 10–15% RH

Variable		<i>O. unguicularis</i>		<i>O. rugatipennis</i>		<i>P. globosa</i>		<i>P. cribripes</i>	
		M	F	M	F	M	F	M	F
Osmolality	\bar{x}	381.6	377.4	390.0	382.0	369.5	378.0	365.8	369.4
(mOsm/kg)	SD	13.7	21.4	19.1	25.4	29.7	13.5	40.2	18.8
Cl	\bar{x}	124.6	120.0	124.4	118.8	116.7	111.4	111.6	115.2
(mmol/l)	SD	18.2	10.5	12.0	7.4	8.1	7.3	12.2	12.1
Na	\bar{x}	114.8†	123.0		114.3†		111.4†	115.8†	134.5
(mmol/l)	SD	10.9	9.9		9.2		8.6	3.0	7.8
K	\bar{x}	14.4	14.7		15.9		16.9	17.4	20.1
(mmol/l)	SD	2.5	1.9		3.6		2.2	2.3	2.4
Body mass*	\bar{x}	536	675	412	786	329	940	917	1300
(mg)	SD	127	139	41	251	40	123	110	181
Weight loss (%)	\bar{x}	5	4	5	4	7	4	6	6
Haemolymph	\bar{x}	99	116	58	161	45	172	174	267
volume (μ l)	SD	51	49	7	46	10	35	62	94
Haemolymph	\bar{x}	18	17	14	21	12	18	19	21
volume (% B.M.)									
N		5	5	5	5	5	5	5	5

*Initial body mass.

†Indicates a significant difference compared to normal values ($P < 0.05$).

principle of the method rests on separation of colloid particles from crystalloid particles by a membrane having an exclusion limit of 15,000 daltons, it is evident that molecules with small molecular masses contribute to the C.O.P. in these insects. These molecules leaked through the membrane and this resulted in the decreased values observed. The results reported here were measured at a standard time of 5 min after introduction of the sample into the colloid osmometer.

The results obtained after dehydration are interesting. It is again clear that no consistent statistically significant interspecies differences were present. The general trend seemed to be one of haemolymph dilution with definite decreases in sodium concentration. Nicolson (1980) showed a similar non-significant decrease in sodium concentration after 2–3 days of dehydration in *O. plana*, as did Hyatt and Marshall (1977) in the cockroach and Nicolson *et al.* (1974) in the stick insect *Carausius*. It is possible that the initial osmoregulatory response to dehydration stress in these beetles is the removal of sodium and addition of water (fat metabolism). These results serve again to emphasize the possible sensitivity of these animals to the external environment as evidenced by the prompt haemolymph compositional changes when confronted with a dehydration stress that is apparently mild (some of these beetles are able to withstand periods of up to 60 days without water).

Table 4. Haemolymph composition in *L. discoidalis* after a fog

Osmolality	\bar{x}	351.4
(mOsm/kg)	SD	28.5
Cl	\bar{x}	113.2
(mmol/l)	SD	9.2
Na	\bar{x}	115.8
(mmol/l)	SD	13.1
K	\bar{x}	18.3
(mmol/l)	SD	0.7
Total protein	\bar{x}	30.1
(g/l)	SD	(pooled)
Haemolymph volume	\bar{x}	39
(μ l)	SD	10
Haemolymph volume		
(% B.M.)		37
N		15

It has been shown that beetles caught after a fog have an increased body mass, that in general, dehydrated animals respond to a fog and hydrated ones do not, that dyed water is found in the intestinal tracts, and that in the case of *L. discoidalis*, water is extracted from the wet sand (Seely and Hamilton, 1976; Hamilton and Seely, 1976). Cooper (1982) observed significant decreases in haemolymph osmolalities following a fog, and this agrees with observations on other tenebrionids in the laboratory (Broza *et al.*, 1976; Coutchie and Crowe, 1979; Nicolson, 1980) and field (Riddle *et al.*, 1976). The results reported here indicate no significant differences between beetles collecting water during a fog compared to those foraging prior to a fog. It is thus difficult to conceive of the former as experiencing a dehydration stress which stimulates them to collect water if available (under certain environmental conditions, e.g. prolonged periods without fogs, this would clearly be the case). One possibility is that animals in need of water do not forage but lie in wait below the surface for a fog. Haemolymph composition from animals caught on the surface prior to a fog would thus not be an indication. Another possibility is that beetles do not dig trenches only because of the need to take in water for osmotic reasons, but also to obtain sufficient fluid volume for e.g. egg and sperm production. No doubt, other possibilities exist (Seely, 1979). The final answer will only be found once haemolymph composition of beetles about to fog bask or trench dig is known (i.e. before precipitation occurs).

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REFERENCES

- Broza M., Borut A. and Pener M. (1976) Osmoregulation in the desert tenebrionid beetle (*Trachyderma philistina* Reich) during dehydration and subsequent rehydration. *Israel J. med. Sci.* **12**, 868–871.
- Cooper P. D. (1982) Water balance and osmoregulation in a free-ranging tenebrionid beetle, *Onymacris unguicularis*, of the Namib Desert. *J. Insect Physiol.* **28**, 737–742.

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- Coutchie P. A. and Crowe J. H. (1979) Transport of water vapour by tenebrionid beetles. II. Regulation of the osmolarity and composition of the haemolymph. *Physiol. Zool.* **52**, 88–100.
- Hamilton W. J. and Seely M. K. (1976) Fog basking by the Namib Desert beetle, *Onymacris unguicularis*. *Nature, Lond.* **262**, 284–285.
- Hyatt A. D. and Marshall A. T. (1977) Sequestration of haemolymph sodium and potassium by fat body in the water-stressed cockroach *Periplaneta americana*. *J. Insect Physiol.* **23**, 1437–1441.
- Lowry O. H., Rosenbrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.
- Nicolson S. W. (1980) Water balance and osmoregulation in *Onymacris plana*, a tenebrionid beetle from the Namib Desert. *J. Insect Physiol.* **26**, 315–320.
- Nicolson S., Horsfield P. M., Gardiner B. O. C. and Maddrell S. H. P. (1974) Effects of starvation and dehydration on osmotic and ionic balance in *Carausius morosus*. *J. Insect Physiol.* **20**, 2061–2069.
- Prather J. W., Gaar K. A. and Guyton A. C. (1968) Direct continuous recording of plasma colloid pressure of whole blood. *J. appl. Physiol.* **24**, 602–605.
- Richardson C. H., Burdette R. C. and Eagleson C. W. (1931) The determination of the blood volume of insect larvae. *Ann. ent. Soc. Am.* **24**, 503–507.
- Riddle W. A., Crawford C. S. and Zeitone A. M. (1976) Patterns of a haemolymph osmoregulation in three desert arthropods. *J. comp. Physiol.* **112**, 295–305.
- Seely M. K. (1979) Irregular fog as a water source for desert dune beetles. *Oecologia (Berl.)* **42**, 213–227.
- Seely M. K. and Hamilton W. J. (1976) Fog catchment sand trenches constructed by tenebrionid beetles, *Lepidochora*, from the Namib Desert. *Science* **193**, 484–486.
- Seely M. K., Lewis C. J., O'Brien K. A. and Suttle A. E. (1983) Fog response of tenebrionid beetles in the Namib Desert. *J. Arid Envir.* **6**, 1–9.

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